REMARKS

I. Status of the Claims

Claims 2, 3, and 6 are amended.

Claim 16 is added.

Claims 9-15 were restricted out reserving the right to prosecute them in a continuing application.

Claims 1, 5, 7, and 8 are canceled.

Claims 2-4, 6, and 16 are pending.

II. Goals of the Invention

A goal of the invention which is to inhibit bacterial IMPDH without inhibiting mammalian IMPDH. This is because required inhibitors of bacterial IMPDH inhibit or kill bacteria that have infected mammalian hosts, without harming the mammalian host. On page one of the specification a goal is given as "elucidation of a kingdom - specific signature for IMPDH enzymes is an important element in the development of specific inhibitors".

The present disclosure relates for the first time, structural differences between bacterial and mammalian IMPDH paving the way toward inhibiting bacteria without inhibiting a mammalian host. *S. pyogenes* was used as a representative sample of bacteria because it is a pathogen, and has only one cysteine at the active site, simplifying some technical steps, and had similar IMPDH amino acid sequences compared to other pathogenic bacteria. A model for a binding pocket, based on *S. pyogenes* will allow finding binding pockets in other bacteria **without undue experimentation**. Sufficient information is provided in the specification. "The invention relates the crystal structure of a bacterial IMPDH with substrate bound in the catalytic site." (Specification page 5, lines 11-12).

The crystal structure shows catalytic site IMP "bound into the pocket located near the surface of the a/β - barrel structure". (Specification page 13, lines 16-22).

One of these sequence regions is the αG helix that forms part of the catalytic pocket. Analysis of sequence alignments for this region (Table 2) indicates a pattern of catalytic residues conserved in all enzymes and a secondary pattern of amino acid conservation associated with either bacterial or eukaryotic IMPDH enzymes." (Specification page 6, lines 1-5).

"....a cofactor plays not only the role of hydride acceptor but also appears to complete the structure of the catalytic pocket." (Specification page 14, lines 18-19).

Identification of differences in the catalytic pocket (binding pocket) between bacterial and mammalian IMPDH provides focus for designing inhibitors of bacteria IMPDH other than *S.*

pyogenes because bacterial species will be more similar to each other than to mammals. Extensive comparisons of IMPDH structure among bacteria and between mammals are disclosed. (Table 2)

On page 8-9, methods are disclosed for determining bacterial IMPDH crystal structure. A map shows a "clearly defined electron density for the IMPDH substrate, bound in the catalytic site," (page 9, lines 22-24). Based on this invention, a model is now available for bacterial IMPDH which provides a framework on which differences among bacteria may be sought.

III. Summary of the Interview of January 15, 2004: Enablement, Written Description

Applicant appreciates Primary Examiner Marschel's comments during the telephone interview of January 15, 2004 with applicant's representative, Alice O. Martin, and an inventor, Dr. Collart.

The prosecution history since the filing date March 23, 2000 was reviewed. Applicants hoped to resolve confusions due to different positions taken by the different examiner's involved in the prosecution.

Dr. Marschel identified one possible source of confusion - whether there was a single species of the IMPDH with a single amino acid sequence, or whether there were multiple species.

In response, Dr. Collart explained that although there are small amino acid sequence differences, the enzyme from a similar organism or the same species, is not highly variant. The overall protein shape (protein fold) is expected to be similar for all bacteria. By definition, all IMPDH enzymes must bind IMP at a specific site defined as the binding pocket. The specification describes an IMPDH protein with IMP localized in the binding site. Furthermore, the inventors describe specific molecular contacts between IMP and the specific amino acids in the protein. Substitution of the contact atoms on IMP is a step for the design of inhibitors. Of course, those skilled in the art could apply more sophisticated methods for drug discovery using the information provided by the structure. Citations to the literature are in the record as evidence that those of skill in the art could design lead compounds based on the present disclosure.

The technique of molecular replacement is well known to those skilled in the art and can be used obtaining initial phasing for an unknown structure from a known, structurally related molecule (J.P. Turkenburg and E.J. Dodson Modern developments in molecular replacement. *Curr. Opin. Struct. Biol.* 1996 Oct;6(5):604-10). When there is a certain level of sequence homology, and the coordinates of the binding pocket of the species is known, 3-D structure is expected to be similar, and an inhibitor that works in the first species is a lead candidate for a inhibitor in a related species. Therefore, Dr. Collart is entitled to claim scope for developing lead compounds in bacteria, not just in *S. pyogenes.*

By definition, all IMPDH enzymes must bind IMP at a specific site defined as the binding

pocket. The specification describes an IMPDH protein with IMP localized in the binding site. Furthermore, the inventors describe specific molecular contacts between IMP and the specific amino acids in the protein (specification page 13, lines 19-32, page 14, lines 1-14 and Figure 3a and b. Substitution of the contact atoms on IMP is a step for the design of inhibitors. Of course, those skilled in the art could apply more sophisticated methods for drug discovery using the information provided by the structure. Whether crystals or high resolution structures are available for other bacterial IMP enzymes (page 4) is not relevant since the invention is enabling for drug design and for comparison with other IMPDH structures should they be available.

The "60% homology" was introduced into claims in response to an examiner's request to exclude human IMPDH, which is known to have less than 60% amino acid sequence homology with IMPDH from bacteria. The definition excludes known mammalian IMPDH enzymes and all eukaryotic IMPDH enzymes for which sequences were available at the time the claims were written. In fact, a basis for the present invention is that inhibiting bacterial IMPDH will not inhibit the human host IMPDH. The cysteine residue in the active site forms a covalent intermediate with IMP. This strategy is clearly proposed in the specification (page 2, 5-15): "This IMPDH consensus region is highly conserved in both bacterial and eukaryotes, with 90% and 85% of the respective residues being identical. However, only 40% of these residues remain identical when compared between the two kingdoms. This limited conservation suggests that bacterial and eukaryotic IMPDH enzymes may have distinct characteristics; a suggestion supported by their kinetic differences and differential sensitivity to inhibitors." (*emphasis provided*) New claim 16 separates out the elements from claim 6.

Dr. Marschel questioned whether the same crystal as disclosed would be produced from the same starting material, using the disclosed methods. Dr. Collart explained that the crystalline form of bacterial IMPDH is reproducible using the methods disclosed in the specification and the starting material, *S. pyogenes*. A Declaration under CFR 1.132 to that effect is appended as Exhibit A. Because of the known amino acid sequence homologs for bacterial IMPDH, the 3-D crystal structure prepared for other bacteria using the disclosed methods, should be the same as that disclosed in the patent application, at least for the binding pocket. The fold of the enzyme won't change. IMPDH is a tetramer with the binding pocket located at the subunit interface. Rearrangements are expected to be changes in the side groups.

Dr. Collart emphasized that the disclosure is directed to methods of developing lead compounds for inhibitors of bacterial IMPDH. However, the intent of the claims is not to teach methods of crystal preparations but to define characteristics of bacterial IMPDH enzymes (and specifically the binding pocket containing IMP). The provision of a crystal is a step toward that

goal. Publications have been provided in the record to attest that those of skill in the art, having crystal coordinates and binding pocket information as result of the present disclosure, can produce lead compounds for inhibitors of IMPDH. The intent of the claims is not to teach methods of crystal preparations but to define characteristics of bacterial IMPDH enzymes (and specifically the binding pocket containing IMP).

During the interview with the examiner, a question regarding the number of IMPDH structures deposited in the PDB was raised. A search of PDB indicates the following inventory of deposited coordinates for IMPDH:

Kingdom	Organism	PDB code(s)
Eucaryotic	*Tritrichomonas foetus*	1AK5, 1LRT, 1ME7, IME8, IME9, IMEI, IMEH, 1MEW, 1PVN
	human	IB30, IJCN, 1NF7, 1NFB
	Chinese hamster	1JR1
Procaryotic	*Streptococcus pyogenes*	1ZFJ
	Borrelia burgdorferi	1EEP

This shows crystals have been obtained repeatedly from the same organism.

Dr. Marschel wondered if the IMPDH had enzyme activity. All agreed that the IMPDH of the present invention has enzyme activity, and that IMP is its target.

IV. Summary of Arguments Against 112 Rejection

The specification clearly provides details on screening procedures used to obtain well diffracting crystals. Making the crystal allowed determination of the atomic coordinates, the binding pocket and other structural features. Because the generation of a crystal is a stochastic process, the methods outlined in the specification represent the accepted approach for the generation of well diffracting crystals. The examiner provides no support for any "undue experimentation" requirement and as the Court in *Wands* stated, "routine experimentation" does not mean it is "undue".

The test [for undue experimentation] is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed to enable the determination of how to practice a desired embodiment of the claimed invention. *Johns Hopkins Univ. v. Cellpro, Inc.*, 152 F.3d 1342, 1360 (Fed. Cir. 1998)(citing *PPG Indus., Inc. v. Gardian Indus. Corp.*, 75 F.3d 1158, 1564, 37 USPQ2d (BNA) 1618, 1623 (Fed. Cir. 1996)).

The present invention is enabling according to *Wands.*

Breadth of Claims: *S. pyogenes* is a model for bacteria based on homology of IMPDH sequences.

Nature of the Invention: Developing lead compounds using *S. pyogenes* binding pocket as a model will be applicable to methods for finding inhibitors for other bacterial IMPDHs.

State of the Prior Art: This is the first disclosure of structural characteristics of a bacterial IMPDH. No other model is available that would lead to binding pockets and inhibitors selective of bacteria.

Level of Ordinary Skill: Publications cited in the disclosure and the exhibits in Responses illustrated the level of skill.

Predictability: Now that a model is disclosed, because of sequence homology and predictable 3-D structures, finding binding pockets to guide development of lead compound requires only routine experimentation.

Direction Provided: The inventor disclosed how to make an IMPDH crystal, to determine an atomic map, and develop lead compounds based on the binding pocket.

Working Examples: *S. pyogenes.*

Quantity of Experiment: The examiner has not demonstrated that more than routine experimentation is required.

The technique of molecular replacement is well known to those skilled in the art and can be used obtaining initial phasing for an unknown structure from a known, structurally related molecule (J.P. Turkenburg and E.J. Dodson Modern developments in molecular replacement. *Curr. Opin. Struct. Biol.* 1996 Oct;6(5):604-10). When there is a certain level of sequence homology, and the coordinates of the binding pocket of the species is known, 3-D structure is expected to be similar, and an inhibitor that works in the first species is a lead candidate for a inhibitor in a related species. Therefore, Dr. Collart is entitled to claim scope for developing lead compounds in bacteria, not just in *S. pyogenes.*

V. Sintchak Does Not Teach IMPDH from a Bacteria

Claim 4 is not anticipated.

Sintchak does not teach bacterial IMPDH. Sintchak teaches mammalian IMPDH from which a distinguishing model is sought in bacteria as disclosed in the pending application. Inhibitors of bacterial IMPDH will not inhibit mammalian IMPDH. "The high-resolution (1.9Å) crystal structure of *S. pyogenes* IMPDH dehydrogenase allows examination of the catalytic site in greater detail than it was possible previously" (specification page 14, lines 9-11). Differences from the structure of bacterial IMPDH from Chinese hamster of Sintchak are described.

> The NAD binding region (between the α_J/β_L loop) was also selected as a target for site-specific mutagenesis. The selection of Glu421 for mutation was based on an analysis of sequence differences at residues corresponding to or near amino acids identified as MPA binding sites in human IMPDH. The conserved glutamate in bacteria is replaced with a conserved glutamine in eukaryotes. This substitution does not alter the apparent activity of *S. pyogenes* IMPDH (Table 6). This result was unexpected since replacement of the corresponding residue in the hamster enzyme (Gln441) with alanine results in a significant decrease in activity (Sintchak *et al.*, 1996).

(specification page 17, lines 10-17).

This structure is significantly more complete (97%) and of higher resolution (1.9Å) than those reported from IMPDH from Chinese hamster (Sintchak *et al.*, 1996) (85%, 2.3Å) and *T. foetis* (Whitby *et al.*, 1997) (specification page 9, lines 4-8 and 19-22).

A comparison amino acid sequences of *S. pyogenes* IMPDH enzymes show only a 35% identity with the Chinese hamster IMPDH of Sintchak. This level of homology did not enable interpretation of the *S. pyogenes* model (specification page 19, lines 24-28). A similar case was observed for the structure of IMPDH from the protozoan (not a bacteria as the examiner mistakenly states on page 14 of the Action) *Tritrichomonas foetus* that shows a similar level of identity to the *S. pyogenes* and Chinese hamster enzymes. If molecular replacement cannot be used for determination of the structure, the information used by Sintchak cannot be used to delineate the structure of the IMPDH enzymes obtained from bacteria. Those of skill in the art know that molecular replacement is generally only applicable for homologues with identity >50%. Homologues with lower identity can not reliably be used to determine structure of homologues used for building of a model as is described in the specification.

VI. Wilson Does Not Teach a Method for Developing a Lead Compound

Claim 4 is not anticipated.

In fact, Wilson relates IMPDH Type II. Mammals have two forms of IMPDH (Type I and II). Bacteria have only a single IMPDH enzyme. There is only 35% identity between the bacterial and both human enzymes making it impossible to use the crystal model of Wilson to solve the bacterial IMPDH molecule structures.

VII. Whitby and Wilson Do Not Make Claim 4 Obvious

Wilson is discussed in Section VI. herein. As discussed before, an inhibitor developed from Whitby would inhibit a mammalian host because it teaches IMPDH from a mammalian host.

VIII. Other Issues

The examiner appeared bothered by the fact that short alignments in specific regions

display higher sequence identity than the binding pocket specification outlined in claims 6 and 7. The binding pocket is an entity with biological significance. Selected excerpts from the sequence may be expected to display a higher sequence identity than longer alignments. This does not negate the significance of the binding pocket. The bottom line is that there are differences in biological properties between prokaryotic/bacterial and eucaryotic enzymes. These must be due to the sequence of amino acids.

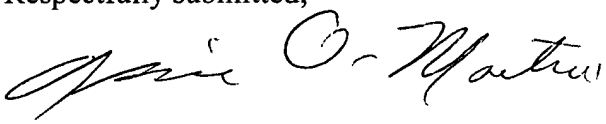
The examiner has a problem with the numbering of residues in the PDB deposit and the alignments represented in Table 7. This is a semantic difference and is not relevant to the merits of the invention. IMPDH enzymes from different species perform the same function but have different amino acid lengths. In the alignment figure, a front to back numbering convention was applied. If the examiner so desires, the numbering system can be altered to correspond to the *S. pyogenes* IMPDH sequence.

• • •

Please contact applicants' representative if you have any questions.

No other fees are believed due at this time, however, please charge any deficiencies or credit any overpayments to deposit account number 12-0913 with reference to our attorney docket number (21416-90042).

Respectfully submitted,



Alice O. Martin
Registration No. 35,601

Dated: February 25, 2004

BARNES & THORNBURG
One North Wacker Drive
Suite 4400
Chicago, IL 60606-2809
(312) 214-8316